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Optimization of Process Variables in the Biodiesel Production from *Lophira lanceolata* Seed Oil

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ABSTRACT
Box-Behnken response surface methodology was employed to optimize the process variables in alkaline-catalysed transesterification of *Lophira lanceolata* seed oil into biodiesel following initial acid-catalysed esterification. The model equation obtained from regression analysis adequately expressed the relationship between the biodiesel yield and the process variables with R^2 of 96.05%.The optimization result suggested the best combination of the process variables for optimum biodiesel yield of 85.0% are: reaction temperature (50^0C); reaction time (120min); amount of methanol (6 cm^3/g oil) and catalyst concentration (0.30 mol/dm^{-3}). Validation yield of 83% compares favorably with the predicted value, showing model reliability in describing the transesterification process. Results of GC-MS analysis of the biodiesel revealed it mainly consists of methyl esters of octadecenoic acid (69.5%), palmitic acid (9.4%) and hexacosanoic acid (5.6%). The biodiesel was very fluid 2.70±0.12 mm/s^2 with most of it fuel properties in conformity with ASTM standards making it suitable as fuel for diesel engines.
Keywords: Optimization, *Lophira lanceolata*, transesterification, Biodiesel, Behnken design.

INTRODUCTION
Biodiesel is a mixture of monoalkyl esters derived from vegetable oils or animal fats. It has properties that are similar to petrodiesel and can thus be used in diesel engines with no engine modification (Atadashi *et al*., 2010). It is renewable, biodegradable, non-toxic and produces much less harmful emissions than conventional petrodiesel (Antolin *et al*., 2002; Issariyakul *et al*., 2008). However, it has slightly lower calorific value than petrodiesel and cost about 1.5 to 3 times higher largely due to high cost of raw materials (Abbaszaadeh *et al*., 2012).

Biodiesel is produced from wide range of feedstock (Fan *et al*., 2010; Gui *et al*., 2008; Karmakar *et al*., 2010) although, for both economics and food security reasons (Wiebe *et al*., 2008), there is more emphasis on non-edible oils (Gui *et al*., 2008; Haas, 2005). Consequently, spent or used oils are gaining attention as low cost raw materials for biodiesel production (Enweremadu and Mbarawa, 2009; Leung and Guo, 2006; Meneghetti *et al*., 2007; Moser, 2008). However, the most promising feedstocks are non-edible oils, such as *L. siceraria* (Muhammad *et al*., 2015), *H. brasiliensi* (Muhammad *et al*., 2016), and *J. curcas* (Koh and Ghazi, 2011; Yang *et al*., 2012).

Nevertheless, irrespective of the source, biodiesel preparation is influenced by a number of factors including reaction temperature, reaction time, methanol/oil ratio and concentration of the catalyst used (Gondra, 2010; Muhammad *et al*., 2016). The optimal levels of these factors are also a function of the nature of oil being esterified (Gondra, 2010; Nkafamiya *et al*., 2010; Singh and Singh, 2009). Hence, transesterification of any given oil needs to be optimized to determine the best conditions for optimal yield. Thus, in this work we used Box-Behnken response surface design to optimize the process variables for conversion of *L. lanceolata* seed oil into biodiesel.

L. lanceolata is a small to medium-sized deciduous ironwood tree (up to 16 m tall and 70cm diameter) with a narrow crown and ascending branches. It is found in savannah in the wild on medium heavy to sandy or gravelly soil.

MATERIALS AND METHODS

Chemicals, solvents and oilseeds

All chemicals and solvents used in this work were of analytical grade manufactured by British Drug House (BDH). These included methanol (99.5%), sodium hydroxide (95%), sulphuric acid (98%) and petroleum ether. The chemicals and solvents were used as procured without further purification.

Seeds of *L. lanceolata* was obtained from the premises of Bauchi Campus of Abubakar Tafawa Balewa University, Bauchi, Nigeria. They were manually separated from the kernels, dried and grounded into powder. Oils were extracted from the grounded seeds by Soxhlet extraction for 6 hours using petroleum ether as solvent (Muhammad *et al*., 2015).

Acid-catalysed esterification

A solution consisting of methanol (2.25g) and sulphuric acid (0.05g) was added to the oil for every 1g of the free fatty acid. The mixture was stirred at 60^{o}C for an hour and allowed to settle for the oil and methanol-water layers to separate. The methanol-water layer was discarded and the bottom oil layer was recovered and analysed for its free fatty acids content. The esterified oil was used in the subsequent experiments.

Design of experiment

Response surface (Box-Behnken) experimental design was used to design the experiments on MINITAB 16 statistical software platform. The aim was to model optimization of transesterification conditions of the oil using methanolic sodium hydroxide as catalyst (Fan *et al*., 2011; Muhammad *et al*., 2015; Muhammad *et al*., 2016). Thus, four independent variables - reaction time, reaction temperature, amount of methanol and catalyst concentration -were investigated and optimized. Table 1 shows the lower and upper levels of the factors employed in the design based on literature survey (Prommuak *et al*., 2012; Silva *et al*., 2011)

Each trial was replicated and all the runs were completely randomized to obtain a total of 58 runs (see Table 2 for the design matrix). The amount of oil was kept constant at 5g for each run.

Description of the experimental trial

In each trial (Table 2), esterified oil (5.0 g) was placed in a round-bottomed flask followed by the addition of appropriate volume (20, 30 or 40 cm^3) of freshly prepared methanolic sodium hydroxide solution of concentration (0.30, 0.475 or 0.65M). The mixture was then refluxed at a constant temperature (40, 50 or 60°C) for a specific period of time (40, 80 or 120 minutes). Thereafter, the flask was allowed to cool and its contents settled. The mixture was then filtered, and the filtrate was transferred into a separating funnel, and then water (10 cm^3) were added to facilitate separation into aqueous and organic layers. The aqueous, glycerol-rich layer was ran off while the organic, biodiesel-rich layer was collected, washed with distilled water and dried over anhydrous sodium sulphate, and finally dried at 60^{o}C to obtain the biodiesel. The percentage of the biodiesel obtained relative to the 5.0 g of the oil was calculated from Equation1 (Muhammad *et al*., 2015; Muhammad *et al*., 2016).

$$Biodiesel\ yield, y\ (\%) = \frac{\text{wt of biodiesel obtained (g)} \times 100}{\text{5g of the oil used}} \quad \text{---------- (1)}$$

Table 1: Independent variables and their levels as used in the Box-Behnken experimental design

SN	Independent variable	Code	Lower level	Mid-level	Upper level
1	Temperature (^{o}C)	*d*	40	50	60
2	Time (minute)	*t*	40	80	120
3	Methanol (cm^3)	*m*	20	30	40
4	Catalyst (M)	*c*	0.300	0.475	0.650

Molecular assay of the biodiesel

A sample of the biodiesel was analysed for its methyl esters composition using a gas chromatography (Agilent 6890N) coupled to mass spectrometer (Agilent 5973 MSD) system equipped with a split injection system. 1µl of the sample was injected into the injector (at 250°C). The column was a DB-1ms capillary column (30m x 320 µm (id), with a film thickness of 1 µm). Helium (1.2 cm^3/min) was the carrier gas. The oven temperature was programmed from 60°C (held for 5 minutes) to 250°C (held for 5 minutes) at a rate of 3°C/min. The chromatogram and mass spectrum of the eluted compounds were recorded on a computer using Chemstation software.

Table 2. The design matrix and results of biodiesel yield from the experimental trials conducted.

Trial	Temp. (0°C)	Time (min)	Catalyst (M)	Methanol (cm^3)	Yield, Actual value (%)	Yield, Predicted value (%)
1	40	40	0.65	30	47	48.27
2	60	40	0.65	30	63	58.55
3	40	120	0.65	30	72	70.78
4	60	120	0.65	30	81	81.06
5	50	80	0.30	20	73	74.93
6	50	80	0.47	20	54	54.55
7	50	80	0.30	40	76	72.88
8	50	80	0.47	40	58	56.53
9	40	80	0.65	20	58	58.83
10	60	80	0.65	20	70	70.65
11	40	80	0.65	40	61	59.17
12	60	80	0.65	40	69	70.24
13	50	40	0.30	30	56	55.95
14	50	120	0.30	30	85	85.45
15	50	40	0.47	30	48	50.88
16	50	120	0.47	30	70	66.38
17	40	80	0.30	30	65	65.19
18	60	80	0.30	30	75	76.21
19	40	80	0.47	30	53	53.87
20	60	80	0.47	30	62	63.40
21	50	40	0.65	20	50	47.23
22	50	120	0.65	20	81	82.26
23	50	40	0.65	40	50	50.23
24	50	120	0.65	40	79	79.18
25	50	80	0.65	30	64	64.67
26	50	80	0.65	30	64	64.67
27	50	80	0.65	30	64	64.67
28	50	80	0.65	30	64	64.67
29	50	80	0.65	30	64	64.67
30	40	40	0.65	30	61	57.32
31	60	40	0.65	30	63	64.43
32	40	120	0.65	30	74	73.83
33	60	120	0.65	30	83	80.94
34	50	80	0.30	20	73	73.88
35	50	80	0.47	20	60	58.50
36	50	80	0.30	40	78	73.29
37	50	80	0.47	40	61	61.94
38	40	80	0.65	20	60	61.86
39	60	80	0.65	20	70	70.51
40	40	80	0.65	40	62	63.66

41	60	80	0.65	40	70	71.57
Table 2 Continued						
42	50	40	0.30	30	60	60.91
43	50	120	0.30	30	85	84.42
43	60	80	0.30	30	67	60.84
44	50	40	0.47	30	71	70.35
45	50	120	0.47	30	68	68.73
46	40	80	0.30	40	77	76.59
48	40	80	0.47	30	60	62.41
49	60	80	0.47	30	67	68.77
50	50	40	0.65	20	51	51.67
51	50	120	0.65	20	84	80.70
52	50	40	0.65	30	55	56.14
53	50	120	0.65	40	78	79.09
54	50	80	0.65	30	68	69.13
55	50	80	0.65	30	68	69.13
56	50	80	0.65	30	68	69.13
57	50	80	0.65	30	68	69.13
58	50	80	0.65	30	68	69.13

Determination of the physicochemical properties

Some physicochemical properties of the biodiesel were determined according to standard procedures: density (ASTM D 5002), kinematic viscosity (ASTM D445), flash point (ASTM D 93), cloud point (ASTM D 2500), pour point (ASTM D 97), acid value (ASTM D 664), ash content (ASTM D 874), and sulphur (ASTM D2622) were determined in triplicates, and the results are presented as the mean ± standard deviation.

Cetane number (*CN*) of the biodiesel was calculated from equation 2 using the saponification value (SV) and iodine value (*IV*)of the biodiesel (Krisnangkura, 1986), and high heating value (HHV) was estimated from equation 3 using viscosity (VS) (Demirbas, 2008)

$$CN = 46.3 + \frac{5458}{SV} - 0.225IV \qquad (2)$$

$$HHV = 0.4625VS + 39.450 \qquad (3)$$

Data analysis

The response, *y* (i.e. biodiesel yield) was fitted with a full quadratic polynomial regression model (eq.4) in order to correlate the yield to the operating variables.

$$y = \beta^0 + \sum_{i=1}^{4} \beta_i \, x_i + \sum_{i=1}^{4} \beta_{ii} \, x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} \, x_i \, x_j \qquad (4)$$

Where β_o β_i, β_{ii}, β_{ij} are intercept, linear, quadratic and interaction coefficients respectively, and x_i andx_j are the independent variables, respectively. The quality of the model fit was evaluated using the coefficient of determination (R^2) and effect of terms was evaluated using ANOVA at α = 0.05. Surface plots were developed using the fitted quadratic polynomial equation obtained from the regression analysis while holding two of the independent variables at constant levels. MINITAB 16 statistical software was used for the data as well as optimization of the results.

RESULTS AND DISCUSSION

The oil yield of the seed was found to be 37.00 ±0.33% with significantly high free fatty acid content of 4.56±0.22 mg NaOH/g oil which necessitated acid-catalysed esterification of the oil before alkaline-catalysed transesterification to avoid soap formation and consequent low yields of the biodiesel (Demirbas, 2003). Following the esterification, free fatty acid determination revealed that the oil had < 0.50 mg KOH/g oil of free fatty acid content, and

therefore suitable for the alkaline transesterification.

The summarized results of the effect of the process variables on the biodiesel yield are presented in Figure 1. It is apparent from the Figure that increase in catalysts conscetration resulted in decrease in the yield followed by slight increase. On the other hand, increase in the methanol and temperature resulted only in slight increase in the yields. A much pronouced increase in yield of the biodiesel was observed with increase in reaction time (Figure 1).

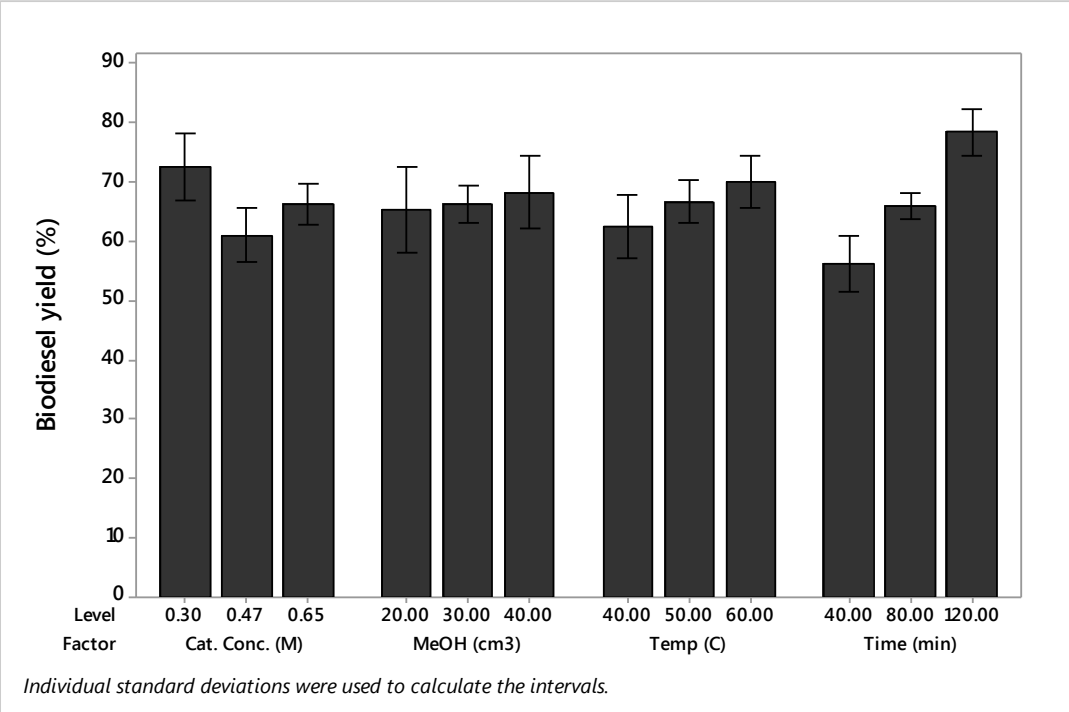

Figure 1: Mean biodiesel yield against levels of the process variables investigated

Process Model Development

Results of Analysis of variance are presented in Table 3. The results reveal that the linear, interaction (2FI) and quadratic terms are significant ($p < 0.05$) while cubic terms are not significant ($p > 0.05$).

Table 4 further shows that with the exception of catalyst concentration (*c*), all the individual effects are significant ($p < 0.05$), and six of the ten interaction terms are also significant ($p < 0.05$). It is noteworthy that all the four interaction terms involving temperature (*d*) are statistically not significant ($p < 0.05$).

The coefficient of determination (R^2) shows that the model, including all the linear and quadratic terms, account for about 96% of the experimental results obtained ($R^2 = 0.9605$) with quite good prediction ability (Pred $R^2 = 0.9106$). On eliminating the terms that are not statistically significant from the model, the final equation (Eq. 5) based on coded terms was obtained.

$$Yield = 61.27 + 3.76d + 10.71t - 1.3m - 6.55c + 9.985t^2 - 2.08c^2 - 6.50dt - 1.50tm - 2.93tc + 17.39mc \quad (5)$$

Table 3. Sequential Model Sum of Squares for *L. lanceolata*

Source	Sum of square	DF	Mean Square	F Value	Prob > F
Mean					
Linear	2.561E+005	1	2.561E+005		
2FI	4593.19	5	918.64	75.21	< .0001
Quadratic	428.61	10	42.86	8.72	< .0001
Cubic	27.91	4	6.98	1.48	0.2262
Residual	118.00	18	6.56	2.16	0.0489
Otal	60.64	20	3.03		

Table 4. Analysis of Variance for Response Surface Model for *Lophira lanceolata*

Source	Sum of Square	DF	Mean Square	F-Value	Prob > F
Model	5021.80	15	334.79	68.08	< 0.0001
Temp. (d)	316.48	1	316.48	64.35	< 0.0001
Time (*t*)	1593.01	1	1593.01	323.93	< 0.0001
Methanol (*m*)	383.35	1	383.35	77.95	< 0.0001
Cat. Conc. (*c*)	19.44	1	19.44	3.95	0.0534
Batch (*e*)	180.40	1	180.40	36.68	< 0.0001
dt	0.000	1	0.000	0.000	1.0000
dm	1.13	1	1.13	0.23	0.6349
dc	2.09	1	2.09	0.43	0.5175
de	15.04	1	15.04	3.06	0.0876
tm	98.00	1	98.00	19.93	< 0.0001
tc	138.33	1	138.33	28.13	< 0.0001
te	54.00	1	54.00	10.98	0.0019
mc	60.95	1	60.95	12.39	0.0011
me	37.50	1	37.50	7.63	0.0085
ce	21.57	1	21.57	4.39	0.0423
Residual	206.55	42	4.92		
Lack of Fit	206.55	34	6.07		
Pure Error	0.000	8	0.000		
Cor Total	5228.34	57			

Effect of the Process Variables of the Biodiesel Yield

Figure 2 to 4 present the contour plots showing effect of various process variables on the conversion of *L. lanceolata* oil into biodiesel as captured by the model (Eq.3). Each plot presents the effect of two variables on the biodiesel yield while holding the other variables at constant level.

Figure 2 shows the interaction between amount of methanol and reaction temperature. Biodiesel yield increases with increase in volume of methanol and reaction temperature while holding reaction time and catalyst concentration at constant levels of 80 minutes and 0.88M, respectively. Evidently, at any given temperature, biodiesel yield can be increased by increasing the amount of methanol, and vice versa. High yields (>64%) are only obtained when > 35 cm^3 of methanol was used when the reaction temperature is above 48°C (Figure 1).This is in agreement with Meher *et al*. (2006) who observed from a survey of literature that for complete transesterification of the oils to be achieved an excess of alcohol is required to drive the reversible reaction to the right for greater conversion in a reasonable time.

Figure 3 shows the contour plot of reaction time against reaction temperature at constant catalyst concentration (0.65 M) and amount of methanol (30cm^3). The plot shows that increase in reaction time and temperature results in

increase in biodiesel yield although the conversion tends to more sensitive to change in reaction time. At any given temperature, the biodiesel yield can be increased to up to over 70% by increasing the reaction time. It is however possible to achieve yields of 64 to 70% with reaction time of less than 70 minutes when the reaction temperature is between 55 to 60°C. Nevertheless, yields above 70% can only be achieved with reaction time of > 105 minutes, irrespective of the reaction temperature.

Figure 4 is a contour plot showing the effect of catalyst concentration and reaction temperature on biodiesel yield at a constant reaction time (120 minutes) and amount of methanol (25. 41cm^3). At catalyst concentration of 0.30 M the biodiesel yield was 81.4% at 40°C, but highest yield of biodiesel was obtained at temperature of 55°C and catalyst concentration of above 0.30M with 88.1% biodiesel yield.

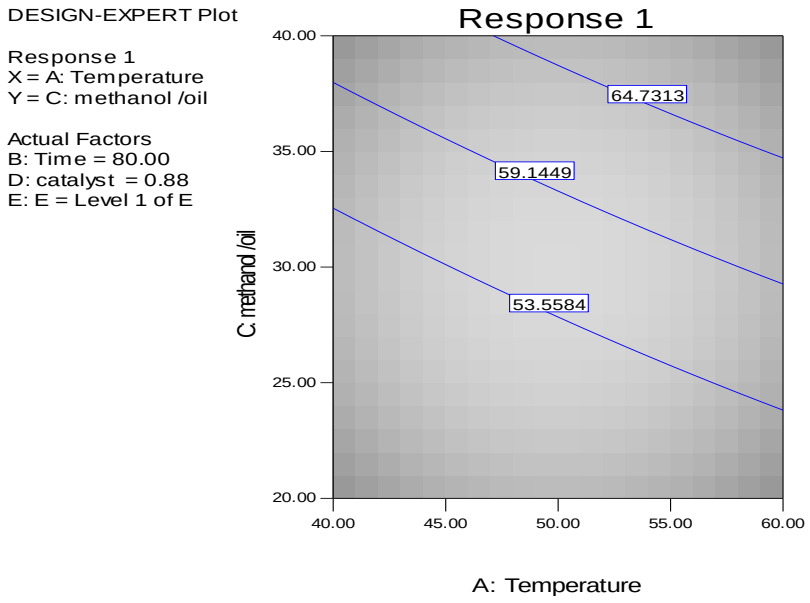

Figure 2: Plot of amount of methanol against reaction temperature for conversion of *L. lanceolate* into biodiesel.

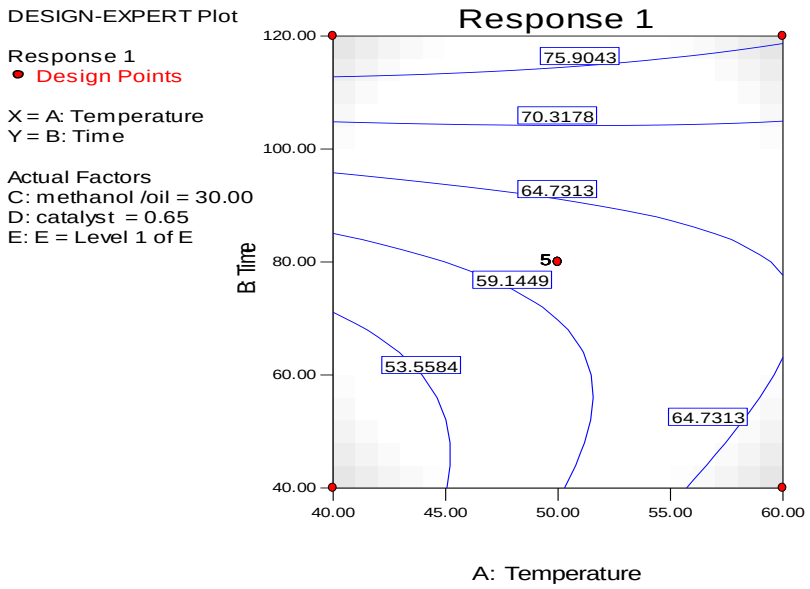

Figure 3: Contour plot of reaction time versus reaction temperature for conversion of *L. lanceolate* into biodiesel.

This suggests that increased in catalyst concentration decreases the conversion rate, but favoured biodiesel yields when temperature increased from 40 to 60°C. In general, high yields can be achieved at longer reaction time irrespective of reaction temperature. It appears that temperature has no major effect on the conversion of vegetable oil into biodiesel as observed by Qian *et al*. (2008).

Figure 4 shows the contour plot of catalyst concentration against reaction temperature at a constant reaction time (120 min) and amount of methanol (25.41cm^3). The figure shows that increase in catalyst concentration appear to decrease the biodiesel yield. Reaction temperature, on the other hand, seems to have little effect on the yield. Highest yields of biodiesel (>75%) can be obtained with catalysts concentration below 0.65M when the reaction temperature is below 59°C.

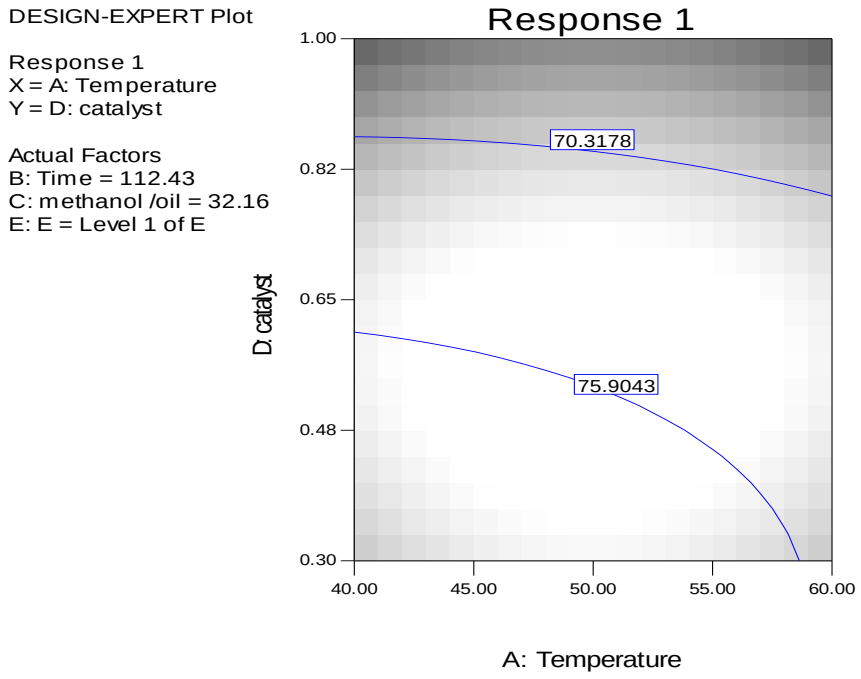

Figure 4: Plot of catalyst concentration versus reaction temperature for conversion of *L. lanceolate* into biodiesel.

Molecular composition of the biodiesel

GC/MS results reveal that the biodiesel from the transesterification of *L. lanceolata* oil consists predominantly of methyl ester of octadecenoic acid (>69%). In general, all the fatty acids methyl ester are either saturated or mono-unsaturated; with no detectable di-, tri- and poly-unsaturated fatty acids. This is a desirable property with respect to the stability of the biodiesel as biodiesels with high degree of unsaturation have been linked to low stability index (Knothe and Dunn, 2003). However, such low levels of unsaturation and relatively high proportion of long chain compounds could also have a negative effect on cold finger plugging point (Ramos *et al*., 2009)and might therefore be responsible for the high levels of cloud and pour points of the biodiesel.

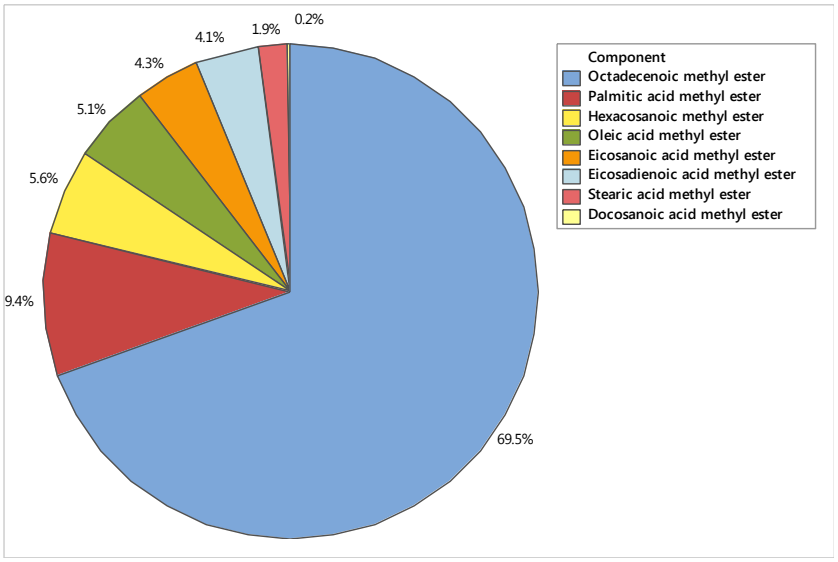

Figure 5: Composition of the biodiesel obtained from the transesterification of *L. lanceolata* seed oil.

Table 5 shows the fuel properties of the biodiesel prepared from the *L. lanceolata* seed oil alongside ASTM D6751 standards for comparison. Notably, the biodiesel has high cetane number compared to biodiesels from many other sources (Bajpai and Tyagi, 2006; Ramos *et al*., 2009) despite its low degree of unsaturation with which it has been observed to correlate positively (Knothe *et al*., 2003; Ramos *et al*., 2009). The relatively high cetane number of the biodiesel may however be due to the significant proportion of methyl ester of the C_{20+} long chain fatty acid acids(Ramos *et al*., 2009). Surprisingly, the biodiesel has quite low viscosity (2.70 mm^2/s, @40°C), similar to Linseed oil biodiesel (Refaat, 2011), despite its high proportion (69.5%) of methyl oleate with viscosity of 4.51 mm^2/s at 40°C (Knothe and Steidley, 2005) coupled with significant amounts of high molecular weight saturated fatty acids methyl ester (Refaat, 2009).Nevertheless, the low viscosity, coupled with relatively low density, is good for atomization in the combustion and could thus reduce formation of soot and engine deposits as may otherwise be suggested by the high ash content of the biodiesel. The relatively high flash point (150°C) reduces risks of fire. The cloud point and pour point are quite high and compares only to tallow and palm biodiesels (Dunn, 2005), which might be a consequence of the high proportion of long chain and saturated fatty acids methyl esters in the biodiesel (Refaat, 2009; Rodrigues *et al*., 2006). Although most of the parameters are within the requirements for good biodiesel, ash content and sulphur content are significantly above the minimum requirements.

Table 5: Fuel properties of the biodiesel obtained from *L. lanceolata* seed oil.

Parameter	Biodiesel	ASTM D6751	EN 14214
Biodiesel content (%)	96.00±5.50	-	96.5 min
Acid value (mgKOH/g)	0.53±0.01	0.5 max	0.5 max
Density (g/cm^3)	0.81±0.03	-	0.86 - 0.90
Viscosity(mm^2/s, @40°C)	2.70±0.12	1.9 - 6.0	2.0 – 5.0
Free glycerol (wt. %)	0.023±0.001	0.02 max	0.02 max
Total glycerol (wt.%)	0.09±0.001	0.25 max	0.25 max

Table 5 Continued

Cetane number	76.74±0.01	47 min	51 min
Flash point (°C)	150.00±5.01	100 min	100 min
High heating value (MJ/kg)	50.99±0.50	-	-
Pour point (°C)	6.0±1.10	-	-
Cloud Point (°C)	18±1.21	Report	-
Ash (%)	0.042±0.001	0.02 max	0.02 max
Sulphur (wt. %)	0.0404±0.00	10 ppm max	10 ppm max

CONCLUSION

The experament results showed that optimal yield (85%) of biodiesel is obtained when transesterification of *L. Lanceolata*seed oil is conducted at at reaction temperature of 50°C, and reaction time of 120minutes when 30 cm^3 (per 5g of oil) of 0.30 M methanolic sodium hydroxide was used 30cm^3. The biodiesel so obtained was found to consist dominantely of octadecanoic acid methyl ester, and was observed be very fluid with most of it fuel properties in conformity with ASTM standards making it suitable as fuel for diesel engines.

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